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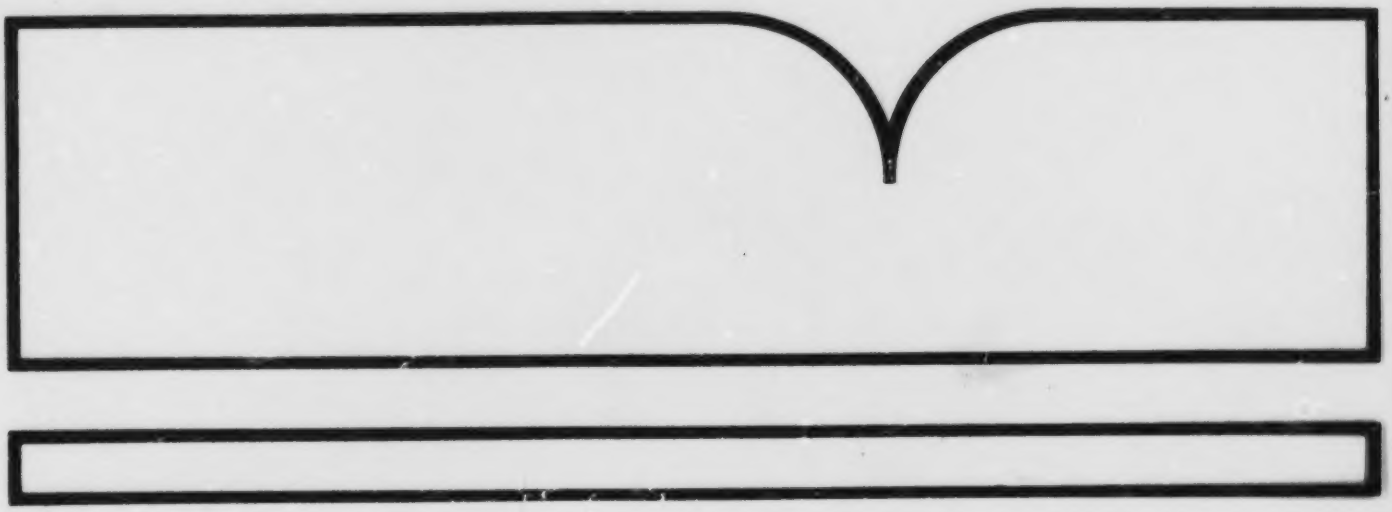

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16. ABSTRACT

Toxicity tests were conducted with selected compounds listed by the United States Environmental Protection Agency (EPA) as priority pollutants. Acute toxicity information was determined for acenaphthene, arsenic trioxide, cadmium chloride, mercury(II) chloride, silver nitrate, chlordane, endosulfan, and heptachlor. Acute tests were conducted using one or more of the following species: fathead minnows (Pimephales promelas), channel catfish (Ictalurus punctatus), rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), bluegills (Lepomis macrochirus), snails (Aplexa hypnorum), or chironomids (Tanytarsus dissimilis). Acute values from these tests ranged from a silver nitrate 96-hr LC50 of 6.7 µg/liter for fathead minnows to an arsenic trioxide 48-hr LC50 of 97,000 µg/liter for chironomids. In addition to acute tests, a fathead minnow embryo-larval exposure was conducted with silver nitrate to estimate chronic toxicity. The estimated maximum acceptable toxicant concentration for silver nitrate, based on fathead minnow survival, lies between 0.37 and 0.65 µg/liter.

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Toxicity of Selected Priority Pollutants to Various Aquatic Organisms

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Toxicity tests were conducted with selected compounds listed by the United States Environmental Protection Agency (EPA) as priority pollutants. Acute toxicity information was determined for acenaphthene, arsenic trioxide, cadmium chloride, mercury(II) chloride, silver nitrate, chlordane, endosulfan, and heptachlor. Acute tests were conducted using one or more of the following species: fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), bluegills (*Lepomis macrochirus*), snails (*Aplexa hypnorum*), or chironomids (*Tanytarsus dissimilis*). Acute values from these tests ranged from a silver nitrate 96-hr LC_{50} of 6.7 μg/liter for fathead minnows to an arsenic trioxide 48-hr LC_{50} of 97,000 μg/liter for chironomids. In addition to acute tests, a fathead minnow embryo–larval exposure was conducted with silver nitrate to estimate chronic toxicity. The estimated maximum acceptable toxicant concentration for silver nitrate, based on fathead minnow survival, lies between 0.37 and 0.65 μg/liter.

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has been ordered by the U.S. Circuit Court to develop water quality criteria for a large number of "priority pollutants." Water quality criteria could not be derived for some chemicals because the data base was inadequate to meet Guideline (Federal Register, 1980) requirements necessary for the derivation of a criterion. The chemicals and test organisms used in the present tests were chosen to fill gaps in the freshwater data bases and ultimately, to enable the derivation of a criterion for these chemicals.

The objective of the test series was to determine acute toxicity information for acenaphthene, arsenic(III) trioxide, cadmium chloride, mercury(II) chloride, silver nitrate, chlordane, endosulfan, and heptachlor using fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), bluegills (*Lepomis macrochirus*), snails (*Aplexa hypnorum*), or chironomids (*Tanytarsus dissimilis*). Individual chemicals were tested with one or more of the test species depending on needs required to meet the freshwater minimum acute data requirements for the derivation of a water quality criterion. In addition to acute tests, a fathead minnow embryo–larval exposure with silver nitrate was conducted.

The eight chemicals tested have freshwater acute toxicity values available in the literature for a variety of aquatic organisms; however, very few or no data were available for the species tested in this study.

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MATERIALS AND METHODS

Exposure Systems

Tests were conducted using static, static with renewal, and flow-through exposure methods (Table 1). Static and renewal chironomid tests were conducted in 90 × 50-cm crystallizing dishes that contained 100 ml of test solution. Renewal tests with chironomids were done by carefully removing all but a few milliliters of the old test solutions and adding new solutions using a 100-ml volumetric pipet. Static and renewal tests with snails were conducted in 2-liter battery jars which contained 1 liter of test solution. Test solutions were poured off and 1 liter of new test solution was added to each of the battery jars during renewal tests with snails. Static fish tests were conducted in 23 × 23-cm stainless-steel tanks containing 9 liters of test solution.

The flow-through exposure system consisted of a modified 2-liter proportional diluter (Mount and Brungs, 1967) with a dilution factor of 0.6. Flow rates and 90% replacement times, determined using the method of Sprague (1969), were 50 ml/min and 5.3 hr for all acenaphthene and cadmium flow-through tests, and 34 ml/min and 8.0 hr for all flow-through silver tests. Glass exposure tanks measured 50.0 × 14.5 × 15.0 cm deep with a 10.0-cm standpipe, which yielded a tank volume of 7.3 liters. Because of the large size of the brook trout, 60 × 30 × 30-cm-deep glass tanks with a test volume of 41 liters were used for the cadmium exposure. A constant 16-hr photoperiod was used during all tests. Fluorescent lamps provided illumination, and light intensity measured 48 L at the water surface.

Physical and Chemical Conditions

All studies were conducted at the Environmental Research Laboratory—Duluth, Duluth, Minnesota. Lake Superior water heated to the appropriate temperature was used in all tests. Test water temperatures were recorded daily (Table 1). Dissolved oxygen (Table 1) was measured using a dissolved oxygen meter (calibrated using the azide modification of the Winkler method) once in one complete set of duplicate tanks (6 tanks) during acute tests and once each week in one set of quadruplicate tanks (6 tanks) during the embryo–larval silver test. All other water quality characteristics were measured using methods described by the American Public Health Association *et al.* (1975). Hardness (EDTA), alkalinity, and pH determinations were made on water samples from three duplicate tanks, including the control and high concentration, once during each acute test and weekly in the embryo–larval test (Table 1). When apparent significant differences were observed between the control and high-exposure tanks, the determinations were repeated on samples from one complete set of duplicate tanks.

Toxicant Solutions and Chemical Methods

The purity of the chemicals tested and the companies which supplied them are given in Table 2. Solvents were used to make superstock solutions for tests conducted with acenaphthene, chlordane, endosulfan, and heptachlor. Acenaphthene was dissolved in isopropanol and 15 ml of this solution was added to the diluter during each cycle. An equal amount of isopropanol was added to each control tank. Isopropanol concentrations were 2550 mg/liter in the control and high-concentration tanks, while the 96-hr LC_{50} for fathead minnows with isopropanol is 10,020 mg/liter (Veith *et*

TABLE 1

TOXICANT, SPECIES, SIZE OR LIFE STAGE, NUMBER OF ORGANISMS EXPOSED PER REPLICATE, EXPOSURE METHOD, FOUR WATER QUALITY PARAMETERS, AND WATER TEMPERATURES DETERMINED DURING THE TESTS

Toxicant	Species	Size or life stage	Number of organisms exposed in each chamber	Exposure method	Dissolved oxygen (mg/l)	Hardness EDTA (mg/l as $CaCO_3$)	Total alkalinity (mg/l as $CaCO_3$)	pH	Temperature (°C)
Acenaphthene	Fathead minnow	0.16 g	25	Flow-through[a]	7.2 (7.0–7.5)[b]	43.3 (42.3–44.2)[b]	41.9 (41.6–42.1)[b]	7.5–7.6[c]	22.9 (22.5–23.2)[b]
Acenaphthene	Channel catfish	5.0 g	10	Flow-through[a]	7.2 (7.0–7.5)	43.3 (42.3–44.2)	41.9 (41.6–42.1)	7.5–7.6	22.9 (22.5–23.2)
Acenaphthene	Rainbow trout	1.3 g	10	Flow-through[a]	10.3 (10.0–10.6)	45.8 (45.6–46.1)	44.1 (43.6–44.6)	7.2–7.4	12.0 (11.8–12.2)
Acenaphthene	Brown trout	0.16 g	10	Flow-through[a]	10.3 (10.0–10.6)	45.8 (45.6–46.1)	44.1 (43.6–44.6)	7.2–7.4	12.0 (11.8–12.2)
Arsenic(III) trioxide	Snail	Adults	10	Flow-through[a]	7.2 (7.0–7.5)	43.3 (42.3–44.2)	41.9 (41.6–42.1)	7.5–7.6	22.9 (22.5–23.2)
Arsenic(III) trioxide	Chironomids	3rd and 4th instar	10	Static	6.9 (6.2–7.8)	49.5 (45.6–53.4)	55.5 (51.0–60.0)	7.4–7.7	25.2 (24.4–26.2)
Cadmium chloride	Brook trout	100 g	10	Flow-through	7.2 (7.1–7.5)	46.6 (44.6–47.5)	36.9 (21.7–47.3)	7.2–7.7	23.8 (23.3–24.2)
Mercury(II) chloride	Snail	Adults	10	Static	10.0 (9.4–11.0)	47.4 (44.2–53.0)	42.9 (42.6–43.6)	7.1–7.2	12.2 (11.7–13.1)
Mercury(II) chloride	Bluegill	0.6 g	10	Static	6.0 (5.5–6.3)	50.0 (45.6–54.3)	37.0 (23.0–51.0)	7.2–7.4	25.1 (24.4–26.2)
Silver nitrate	Fathead minnow	0.15 g	25	Flow-through	4.1 (2.9–6.4)	45.6 (45.6–45.6)	46.5 (42.0–51.0)	7.1–7.3	24.8 (23.5–26.2)
Silver nitrate	Channel catfish	14.2 g	5	Flow-through	6.9 (6.7–7.1)	44.4 (44.1–44.6)	40.6 (38.9–41.4)	7.2–7.3	23.1 (22.4–23.4)
Silver nitrate	Snail	Adults	10	Static (24-hr renewal)	4.2 (4.0–4.3)	44.4 (44.1–44.6)	40.6 (38.9–41.4)	7.2–7.3	23.1 (22.4–23.4)
Silver nitrate	Fathead minnow	0.15 g	10	Static	Not measured	50.4 (49.5–51.9)	44.7 (40.4–48.3)	7.5–7.5	25.5 (22.5–26.5)
Chlordane	Fathead minnow	—	50/25[d]	Embryo-larval	6.2 (6.0–6.6)	44.8 (44.6–45.0)	40.8 (39.0–42.6)	7.2–7.3	23.5 (23.5–23.5)
Chlordane	Snail	Adults	10	Static[a]	7.5 (6.6–8.1)	45.1 (44.6–46.1)	42.6 (41.4–43.8)	7.2–7.7	25.0 (23.6–25.8)
Endosulfan	Chironomids	3rd and 4th instar	10	Static[a]	5.0 (4.3–5.4)	51.0 (48.0–56.3)	89.6 (61.6–134)	7.5–7.6	24.8 (23.8–27.0)
Heptachlor	Snail	Adults	10	Static (24-hr renewal[a])	8.3 (8.2–8.5)	51.2 (49.7–52.4)	49.2 (48.5–49.6)	7.3–7.5	24.8 (23.8–27.0)
Heptachlor	Snail	Adults	10	Static (12-hr renewal[a])	Not measured	48.8 (47.5–50.4)	55.9 (49.3–59.6)	7.1–7.2	24.5 (24.2–24.8)
	Chironomids	3rd and 4th instar	10	Static (12-hr renewal[a])	8.0 (7.9–8.1)	44.6 (44.1–45.6)	41.7 (41.4–42.4)	7.5–7.5	24.4 (23.3–25.4)
					8.0 (7.9–8.1)	44.6 (44.1–45.6)	41.7 (41.4–42.4)	7.4–7.5	24.4 (23.3–25.4)

[a] A solvent was used to solubilize the compound.
[b] Mean and range.
[c] Range.
[d] 50 embryos and 25 larvae were exposed in each quadruplicate tank.

al., 1983). Chlordane, endosulfan, and heptachlor were dissolved in dimethyl formamide (DMF) with equal amounts of DMF (0.5 ml/liter) added to all static test concentrations and controls. There were no observable adverse effects or mortalities in control organisms exposed to the concentrations of isopropanol or DMF that were used.

No water concentration measurements were made during the mercury(II) chloride static tests; LC_{50} values in these tests are reported on a nominal concentration basis. All other LC_{50} values presented are reported as measured concentrations. Water samples were analyzed for toxicant concentrations once during each chlordane test and the arsenic snail test. Water samples were analyzed twice in the arsenic chironomid test, the endosulfan test, the silver snail test, and both heptachlor tests. Daily analyses were made in all flow-through acute tests with acenaphthene, cadmium, and silver, and in the static silver test with fatheads. Water samples were analyzed twice each week during the silver embryo–larval test.

The chemical analyses for arsenic, cadmium, and silver were performed by atomic absorption spectrophotometry. A Perkin–Elmer 5000 spectrophotometer equipped with an HGA 500 graphite furnace was used for samples in the microgram per liter concentration range, while a Perkin–Elmer 403 equipped with an air–acetylene flame was used for samples in the mg/liter range. Samples were acidified to 0.5% with concentrated nitric acid and analyzed by comparison to a standard curve of known concentrations. Spike recoveries for arsenic and cadmium were 91 ± 0% (n = 1) and 99 ± 3% (n = 4), respectively. Final concentrations were not corrected for recovery. Silver was analyzed by a method of internal standardization and no recoveries were performed.

Chlordane, endosulfan, and heptachlor were analyzed by gas chromatography using a Tracor 550 gas chromatograph equipped with a Hewlett–Packard 7670A automatic sampling system. Water samples (50 ml) were added to 100-ml volumetric flasks

TABLE 2

TEST COMPOUNDS, PURITY, AND SUPPLIER OF COMPOUNDS FOR TESTING

Chemical name	Purity or grade	Chemical supplier
Acenaphthene	99%	Aldrich Chemical Co., Milwaukee, Wisc. 53233
Arsenic (As_2O_3)	97+%	Ventron–Alpha Inorganics, Beverly. Mass. 01915
Cadmium ($CdCl_2$)	ACS grade-99%	Matheson, Coleman and Bell, Norwood, Ohio 45212
Mercury ($HgCl_2$)	99+%	Matheson, Coleman and Bell, Norwood, Ohio 45212
Silver ($AgNO_3$)	99.99%	Research Organic/Inorganic Chemical Corp., Sunvalley, Calif. 91352
Chlordane[a]	43%	Velsicol Chemical Corp., Chicago, Ill. 60611
Endosulfan (Thiodan-2)[a]	23.5%	Chevron Chemical Co., Ortho Div., San Francisco, Calif. 94119
Heptachlor[a]	74%	Velsicol Chemical Corp., Chicago, Ill. 60611

[a] Concentrations reported in μg/l of the technical-grade material.

containing 50 ml of hexane. The samples were then stirred vigorously on magnetic stirring devices for 1.5 hr and allowed to separate for 1 hr. The samples were diluted with hexane, if necessary, and analyzed by comparison to known hexane standards. Known amounts of the chemicals were added to water samples to determine the efficiency of this extraction procedure and the spike recoveries were 97 ± 5% (n = 2), 103 ± 0% (n = 1), and 105 ± 11% (n = 6) for chlordane, endosulfan, and heptachlor, respectively. Final concentrations were not corrected for recovery and are reported as micrograms/liter of the technical-grade substances to be consistent with values previously reported in the Criteria Documents (*Federal Register*, 1980).

Acenaphthene was analyzed using a Baird Atomic Ratio recording spectrofluorimeter (Model SFR 100). The wavelength settings were 290 nm for excitation and 336 nm for emission. The slitwidths used were 10 nm for excitation and 20 nm for emission. Isopropanol was added to samples and standards to make a 25% solution and quantitation was accomplished by means of a standard curve. Known amounts of acenaphthene were added to control water and the mean recovery was 92 ± 6% (n = 14). Final concentrations were not corrected for recovery.

Biological Procedures—Acute

Acute fathead minnow tests were conducted using 32- or 33-day-old fish. Fatheads, chironomids, and snails were obtained from stock-culture units of the Environmental Research Laboratory—Duluth. Minnow and snail larvae were kept in a rearing system at 25°C and were fed live brine shrimp until used for testing. Juvenile rainbow trout were obtained from Fattig Hatcheries, Brady, Nebraska, while brown trout and brook trout were obtained as eyed eggs from the Crawford National Fish Hatchery, Crawford, Nebraska. Trout were held in 530-liter fiberglass holding tanks at 12°C and fed a commercial formulation of dry food until used for testing. Bluegills were obtained from Lake Mills National Fish Hatchery, Lake Mills, Wisconsin, and channel catfish were obtained from Senecaville National Fish Hatchery, Senecaville, Ohio. Bluegills and catfish were held in 530-liter fiberglass holding tanks at 23°C and fed a commercial dry food supplemented with frozen adult brine shrimp until used in tests.

The number of organisms exposed in each tank and the life stage or size of the organisms used during each test are presented in Table 1. Chironomids were individually counted into crystallizing dishes containing food and a sand substrate as recommended by Anderson (1980). After allowing 24 hr for the larvae to build a tube, the water containing the food was carefully drawn off and replaced with a randomly assigned exposure concentration. Snails were counted into 10 × 3-cm-wide stainless-steel screen cylinders which were closed on one end by stainless-steel screen and on the other end by a No. 7 neoprene stopper. The cylinders were then randomly distributed to the exposure tanks. Groups of fish were counted into plastic freezer containers and were randomly assigned to the exposure tanks. Fish were not fed 24 hr before or during a test. Average weights of exposed fish (Table 1) were obtained by weighing subsamples of 25 fathead minnows and 20 individuals of all other fish species tested. Death was the endpoint in all acute tests. Dead fish were counted and removed three times on the first day and twice daily thereafter. Chironomid and snail mortalities were counted at the end of the respective 48- and 96-hr tests. Other acute procedures or methods not specified followed those described by the U.S. Environ-

mental Protection Agency Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

Embryo–Larval

Fathead embryos were obtained from the stock culture unit from which fathead minnows for acute tests were obtained. The test was begun by randomly distributing groups of 50 embryos (<24-hr-old) to each of the 24 exposure tanks. Embryos were incubated in oscillating cups made from 120-ml glass jars with 40-mesh nylon screen bottoms. Nonviable eggs were removed daily by observation under a dissecting microscope until hatching was complete. At complete hatch, the number of normal larvae were recorded and 25 normal larvae from each cup were placed in their respective tanks for the larval exposure. Larvae that were dead, obviously deformed, or suffering from a severe pericardial edema were considered as abnormal. Equal amounts of live brine shrimp (<12-hr-old) were distributed to all tanks three times a day Monday through Friday and once a day on weekends throughout the test. Survival counts were made at 96 hr, and weekly thereafter. Surviving fish 28 days after hatch were killed in ice water, blotted dry, and weighed to the nearest milligram.

Statistics

Determinations of LC_{50} values were made using a computerized Trimmed–Spearman–Karber method (Hamilton *et al.*, 1977) on combined mortality data from the duplicate exposure tanks.

Percentage hatchability and percentage survival data from the embryo–larval test were normalized using an arcsin–square root transformation procedure described by Bartlett (1947). Normalized hatchability and survival data and growth data were subjected to one-way analysis of variance ($P = 0.05$) and to subsequent analysis with Dunnett's one-sided comparison of treatment means to control means ($P = 0.05$) (Steel and Torrie, 1960). Growth data of individual fish from each series of quadruplicate tanks were combined before conducting the analysis of variance.

RESULTS

Acute

Acute tests with acenaphthene and four fish species gave 96-hr LC_{50} values that ranged from 580 μg/liter for brown trout to 1720 μg/liter for channel catfish (Table 3). Of the eight chemicals tested, silver nitrate was the most toxic chemical to all tested organisms and was approximately twice as toxic to fathead minnows, the most sensitive species tested, under flow-through conditions than under static conditions (Table 3). Fish exposures to other metals gave 96-hr LC_{50} values which varied from 160 μg/liter for mercuric chloride vs bluegills to 5080 μg/liter for cadmium chloride vs brook trout. Arsenic trioxide was the least toxic chemical tested to snails (96-hr LC_{50} of 24,500 μg/liter) and to chironomids (48-hr LC_{50} of 97,000 μg/liter). Snail exposures to other chemicals gave 96-hr LC_{50} values that ranged from 241 μg/liter for silver nitrate to 1450 μg/liter for heptachlor. A chironomid exposure to chlordane gave a 48-hr LC_{50} of 1440 μg/liter. No mortalities were observed during snail tests with acenaphthene and endosulfan, or during the chironomid test with heptachlor. The LC_{50} values for these chemicals are therefore reported in Table 3 as greater than the highest exposure concentration. The 24-, 48-, 72-, and 96-hr LC_{50} values for all

TABLE 3

THE 24-, 48-, 72-, AND 96-hr LC_{50} VALUES EXPRESSED AS μg/liter FOR THE VARIOUS TESTED PRIORITY POLLUTANTS

Toxicant	Species	Test method[a]	24-hr LC_{50}	48-hr LC_{50}	72-hr LC_{50}	96-hr LC_{50}
Acenaphthene	Fathead minnow	FT[b]	—[c]	—[c]	1700 (1610–1780)[d]	1600 (1560–1630)
Acenaphthene	Channel catfish	FT[b]	—[c]	—[c]	—[c]	1720 (1570–1880)
Acenaphthene	Rainbow trout	FT[b]	1570 (1330–1850)	1130 (1010–1270)	800 (710–900)	670 (600–750)
Acenaphthene	Brown trout	FT[b]	840 (750–950)	650 (590–720)	600 (530–670)	580 (510–660)
Acenaphthene	Snail	FT[b,e]	—	—	—	>2040
Arsenic trioxide	Snail	S[e]	—	—	—	24500 (21500–28000)
Arsenic trioxide	Chironomids	S[f]	—	97000 (95100–99000)	—	—
Cadmium chloride	Brook trout	FT[g]	—[c]	6160 (5200–7300)	5240 (4440–6190)	5080 (4320–5970)
Mercury(II) chloride	Bluegill	S[h]	280 (220–370)	250 (210–300)	160 (140–190)	160 (130–180)
Mercury(II) chloride	Snail	S[e,h]	—	—	—	370 (330–420)
Silver nitrate	Fathead minnow	FT[g]	12.9 (11.8–14.0)	9.5 (8.7–10.4)	7.2 (6.8–7.7)	6.7 (6.4–7.0)
Silver nitrate	Fathead minnow	S[g]	14.0 (12.8–15.3)	14.0 (12.8–15.3)	14.0 (12.8–15.3)	14.0 (12.8–15.3)
Silver nitrate	Channel catfish	FT[g]	—[c]	—[c]	18.4 (15.4–22.0)	17.3 (15.5–19.2)
Silver nitrate	Snail	R[e,g]	—	—	—	241 (162–357)
Chlordane	Snail	S[b,e,i]	—	—	—	1250 (1090–1430)
Chlordane	Chironomids	S[b,f,i]	—	1440 (1200–1730)	—	—
Endosulfan	Snail	R[b,e,i]	—	—	—	>1890
Heptachlor	Snail	R[b,e,i]	—	—	—	1450 (1260–1680)
Heptachlor	Chironomids	R[b,f,i]	—	>2500	—	—

[a] FT = flow-through, S = static, R = renewal.
[b] A solvent was used to solubilize these toxicants.
[c] No LC_{50} values could be determined because of insufficient mortality in the highest exposure concentrations.
[d] These LC_{50} values represent the combined duplicate tank mortality with the 95% confidence intervals in parentheses.
[e] Only 96-hr mortality counts were made with snails.
[f] Only 48-hr mortality counts were made with chironomids.
[g] LC_{50} values were calculated using micrograms per liter of the active ingredient.
[h] The LC_{50} values reported for mercury(II) chloride were based on nominal concentrations.
[i] LC_{50} values were calculated using concentrations of the technical grade material.

species of fish, 48-hr LC_{50} values for chironomids, and 96-hr LC_{50} values for snails for all chemicals tested are given in Table 3.

Embryo–Larval

Exposure of fathead minnows to silver nitrate significantly reduced the percentage of normal larvae at hatch, survival, and growth of the test fish. The percentage of normal larvae at hatch was significantly reduced at silver concentrations ≥ 1.80 μg/liter, survival was significantly reduced after 28 days of exposure to silver concentrations of ≥ 0.65 μg/liter, and growth of 28-day-old fish was significantly reduced at silver concentrations of ≥ 1.07 μg/liter (Table 4). The estimated maximum-acceptable toxicant concentration (MATC) for silver nitrate, based on fathead minnow survival, lies between 0.37 and 0.65 μg/liter.

DISCUSSION

Various test methods were used for conducting acute tests in the present study. The Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses (*Federal Register*, 1980) has approved all of the methods used in the present tests in the determination of a criterion for a chemical. The methods used were chosen because of their expediency and ability to produce valid toxicity values. Most stable compounds which did not degrade and which were not volatile were tested under static conditions. Chemicals that were suspected to degrade were tested using static methods with either 12- or 24-hr renewal of the toxicant solutions. Metal salts such as cadmium chloride and silver nitrate were easily dissolved in water and were for the most part conducted under flow-through conditions. Acenaphthene, which was found to degrade very rapidly during the first day of testing under static conditions, was also tested using flow-through methods.

The present tests involved the use of solvent carriers (DMF and isopropanol) to help solubilize the pesticides (chlordane, endosulfan, and heptachlor) and acenaphthene into superstock solutions. There is a possibility that test concentrations may have exceeded the water solubility of chemicals in tests conducted with solvent carriers.

TABLE 4

NORMAL LARVAE AT HATCH, SURVIVAL, AND GROWTH OF FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF SILVER NITRATE FOR 28 DAYS

Measured concentration (μg/l)	Normal larvae at hatch (%)	Survival (%)	Average weight of all fish (mg)
Control, not detectable	92.0 ± 5.2[a,b]	92.0 ± 13.5[a,c]	165.5 ± 52.1[a]
0.37 ± 0.11[a]	92.5 ± 4.1	83.0 ± 6.0	179.9 ± 49.0
0.65 ± 0.08	95.0 ± 2.6	77.0 ± 8.9[d]	158.2 ± 56.6
1.07 ± 0.17	87.0 ± 8.7	14.0 ± 14.8[d]	101.1 ± 41.2[d]
1.80 ± 0.33	1.5 ± 3.0[d]	1.0 ± 2.0[d]	53.0 ± 0[d]
3.29 ± 0.43	0 ± 0[d]	0 ± 0[d]	0 ± 0[d]

[a] Mean ± SD (data from quadruplicate tanks combined).
[b] Two hundred eggs exposed per concentration.
[c] Survival based on 100 larvae per concentration.
[d] Values significantly ($P = 0.05$) less than controls.

When water solubility is exceeded, it is unknown what portion, if any, of the chemical present as a suspension or in a colloidal state is available to the test organism and how this, in turn, affects toxicity. An attempt was made to not exceed the known water solubility in cases where water solubility information was available for these chemicals. However, with several pesticides, water solubility may have been exceeded in trying to achieve levels that produced mortalities in tested organisms. The highest concentrations tested with heptachlor (2500 μg/liter) apparently exceeded solubility since these mixtures appeared slightly clouded when mixed.

There is a large amount of acute toxicity data on a variety of aquatic organisms, as well as some chronic toxicity information available in the literature for the chemicals tested. Previously available information for these chemicals has been documented in the respective Ambient Water Quality Criteria Documents (*Federal Register,* 1980).

Limited acute data are available in the literature for acenaphthene. The 96-hr LC_{50} of 1700 μg/liter determined for bluegills by the USEPA (1978) compared quite closely with 96-hr LC_{50} values calculated for fathead minnows (1600 μg/liter) and channel catfish (1720 μg/liter) in the present tests. Since acenaphthene was less toxic to snails (no mortality at 2040 μg/liter) than any of the tested fish species, this test indicates that snails were not among the most sensitive species to this chemical.

The present tests indicated that snails were tolerant to chlordane, endosulfan, and heptachlor and that chironomids were tolerant to chlordane and heptachlor. The endosulfan value determined for snails (>1890 μg/liter) and the heptachlor value determined for chironomids (>2500 μg/liter) indicate that these species were not among the most sensitive species to these chemicals. The highest acute values reported in the literature for these pesticides during previous tests with aquatic species were 190 μg chlordane/liter with guppies, *Poecilia reticulata,* (Henderson *et al.,* 1959); 740 μg endosulfan/liter with the cladoceran, *Daphnia magna* (Lemke, 1981); and 320 μg heptachlor/liter with goldfish, *Carassius auratus* (Henderson *et al.,* 1959).

Snails (96-hr LC_{50} of 24,500 μg/liter) and chironomids (48-hr LC_{50} of 97,000 μg/liter) were also found to be quite tolerant to arsenic trioxide. Acute LC_{50} values for other aquatic organisms and for sodium arsenite in the literature range from 812 μg/liter for cladocerans, *Simocephalus serrulatus* (Sanders and Cope, 1966), to 41,760 μg/liter for bluegills (Cardwell *et al.,* 1976).

Carroll *et al.* (1979) determined static 96-hr LC_{50} values for cadmium sulfate vs brook trout that ranged from 2.4 to 29.0 μg/liter depending on water hardness. These values are much lower (possibly due to age and size of exposed trout) than the cadmium chloride flow-through 96-hr LC_{50} of 5080 μg/liter for brook trout in the present test.

Mercury(II) chloride acute values available in the literature for aquatic organisms range from 5 μg/liter for *D. magna* (Biesinger and Christensen, 1972) to 2000 μg/liter for stoneflies (*Acroneuria lycorius*), mayflies (*Ephemerella subvaria*) and caddisflies (*Hydropsyche betteni*) (Warnick and Bell, 1969). Snails were about 2.3 times more tolerant than bluegills to mercury(II) chloride in the present test, although LC_{50} values for both species were within the range of acute values mentioned previously.

Ninety-six-hour LC_{50} values of 6.7 μg/liter in a flow-through test and 14.0 μg/liter in a static test were determined for silver nitrate vs fathead minnows. These values compare closely with soft water 96-hr LC_{50} values for silver vs fathead minnows (3.9–

30.0 μg/liter) obtained by Lemke (1981) who also determined that the fathead minnow 96-hr LC_{50} values for silver ranged from 110 to 270 μg/liter in hard water.

Davies *et al.* (1978) determined an estimated MATC of 0.09–0.17 μg/liter, based on reduced survival of rainbow trout during a silver embryo–larval test. The estimated silver MATC for fathead minnows, also based on survival, was between 0.37 and 0.65 μg/liter in the present test. This indicates that rainbows are slightly more sensitive to silver than are fathead minnows.

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